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Transplacental Transmission of Human Polyomavirus BK

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The presence of BK virus (BKV) and JC virus (JCV) in autopsy materials (placenta, brain, and kidney) of aborted fetuses was investigated by PCR using two sets of primers, specific for the regulatory region (RR) and for the capsid protein VP1, respectively. The RR of BKV was detected in 12 samples of placenta and brain and in nine samples of kidney obtained from 15 fetuses. Out of the 12 positive cases, four placentas, one brain, and three kidney samples also showed the presence of BKV DNA in the VP1 region. Of 12 placentas from a control group with a normal pregnancy outcome, the RR of BKV was detected in six samples, four of which were also positive for the VP1 region. None of the samples from either group was positive for the RR of JCV. In two cases, the nucleotide sequence of the BK RR demonstrated that the viruses isolated from maternal and fetal tissues showed a high homology with one another and had a characteristic deletion of the R_{63} box compared to the archetype strain. The results indicate that BKV may be transmitted vertically. ***J. Med. Virol. 56:372–376, 1998.***

KEY WORDS: human polyomavirus; vertical transmission; regulatory region sequence

INTRODUCTION

Human polyomavirus BK (BKV) was first isolated in 1971 from the urine of an immunocompromised renal transplant patient in England [Gardner et al., 1971]. At the same time, another human polyomavirus, JC virus (JCV), was recovered from the brain of an American patient with Hodgkin's disease and progressive multifocal leucoencephalopathy (PML) [Padgett et al., 1971].

Both BKV and JCV circulate in a large proportion of the population worldwide [Brown et al., 1975]. Serological studies have shown that antibodies to BKV and JCV are present in 80% and 70% of adults, respectively [Gardner, 1973; Brown et al., 1975; Flaegstad et al., 1988; Sundsfjord et al., 1990].

BKV infection is common in early childhood, with a peak incidence between the ages of two and five years. The majority of children experience their primary infection before starting school [Jin et al., 1995; Di Taranto et al., 1996]. Passively acquired maternal antibody is present at birth and declines clearly in the first three months of life. Although maternal antibody may mask active infection, primary BKV infections are probably uncommon before the age of 12 months [Gardner, 1973].

Primary infection with BKV or JCV is usually subclinical and is only occasionally associated with upper respiratory or urinary tract disease. Following primary infection, the viruses remain latent in the kidney [Yoshiike and Takemoto, 1986]. In later years, reactivation of BKV and JCV may occur in healthy individuals but it is more frequent in immunocompromised groups, especially when T-cell functions are depressed [Coleman et al., 1983; Gardner et al., 1984; Flaegstad et al., 1988; Jin et al., 1995]. BKV has been demonstrated in individuals after kidney and bone marrow transplantation and in pregnant women due to alterations in the immune system and possibly hormonal changes in pregnancy [Coleman et al., 1980; Jin et al., 1995]. Several studies have shown that pregnant women have increased susceptibility to infections or reactivation of latent virus such as BKV and JCV [Coleman et al., 1983].

The demonstration of BK virus-specific IgM antibod-

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TABLE I. Oligonucleotide Primers Used in This Study

Region	Sense	Position (nt)[a]	Sequence (5′–3′)
VP1 (VP1-7)	(+)	1480–1500 (BKV)	ATCAAAGAACTGCTCCTCAAT
VP1 (VP1-2R)	(–)	2038–2059 (BKV)	GCACTCCCTGCATTTCCAAGGG
VP1 (327-1)	(+)	1630–1649 (BKV)	CAAGTGCCAAAACTACTAAT
VP1 (327-2)	(–)	1937–1956 (BKV)	TGCATGAAGGTTAAGCATGC
RR (BKTT1)	(+)	5106–5133 (BKV/JCV)	AAGGTCCATGAGCTCCATGGATTCTTCC
RR (BKTT2)	(–)	629–657 (BKV/JCV)	GCTGCTCTAGCACTTTTGGGGGACCTAG
RR (JC1)	(+)	238–263 (JCV)	AGCCTGGTGACAAGCCAAAACAGCTCT
RR (JC2)	(–)	–45–21 (JCV)	CCTCCACGCCCTTACTACTTCTGAG
RR (BK1)	(+)	391–415 (BKV)	GTTCTGCGCCAGCTGTCACGACAAG
RR (BK2)	(–)	–85–49 (BKV)	GGCCTCAGAAAAAAGCCTCCACACCCTTACTACTTGA

[a]Numbers of BKV are from Seif et al. [1979] and those of JCV are from Frisque et al. [1984].

ies in children less than 2 weeks of age suggested prenatal infection with BK virus [Rziha et al., 1978b]. Possible transplacental transmission was supported by data reporting BK virus-specific IgM antibodies in 7.5–9.1% of umbilical cord blood sera from over 800 babies [Rziha et al., 1978a; Taguchi et al., 1975]. However, the virus has not been isolated from fetal kidneys or from placenta, amniotic fluid, or neonatal urine [Coleman et al., 1980], although none of these has yet been studied by more sensitive polymerase chain reaction (PCR) techniques.

Our study was carried out by amplifying the viral DNA in autopsy samples (placenta, brain, and kidney) of aborted fetuses and in placentas of women with a normal pregnancy outcome in order to provide evidence of the presence of polyomaviruses in placenta and in fetal organs. The purpose of this study was to elucidate a possible new route of polyomavirus transmission.

MATERIALS AND METHODS

Samples

Autopsy materials including placenta, brain, and kidney were collected from 15 aborted fetuses, 10 of which had been fixed previously and embedded in paraffin. The pregnancies had been terminated between weeks 16 and 24 for the following reasons: nine cases presented with encephalic malformation (six hydrocephalus and three Arnold-Chiari disease), two had renal malformation (polycistic kidney), three presented a trisomy 18, and one presented a trisomy 21.

No morphological abnormalities were found in maternal placenta or umbilical cord by gross inspection, but subchorionic fibrin plaques and foci of intervillous fibrin deposition were found microscopically. The histological findings did not show any characteristics of viral lesions.

Kidneys and brains were collected with a Rokitansky technique and the samples were taken from the inner most part of the organ in order to eliminate any possible contamination. Three-μm sections were prepared and stained with hematoxylin and eosin. All study procedures were developed and carried out in accordance with current standards for ethical scientific research. Non-embedded placental samples from 12 mothers with normal pregnancy outcome were tested in parallel.

DNA Extraction

Tissue fragments were cut and minced with a single disposable scalpel. From paraffin-embedded specimens, sections of 2–5 μm were cut and deparafinized with xylol/ethanol [De Mattei et al., 1995].

All the samples were submitted to a rapid DNA extraction by QIAamp Tissue Kit (M-Medical-Genenco, Qiagen, Santa Clarita, CA). DNA yield was determined by measuring its concentration by absorbancy at 260 nm and then 1 μg of total DNA was directly used in PCR amplification.

Conditions for PCR Amplification

Each DNA sample was first checked for suitability for PCR analysis by PCR amplification of HLA gene sequences using primers GH26 and GH27 for HLA Dq Alpha locus (Synthetic Genetics, San Diego, CA). Only positive samples were further investigated for amplification of the target DNA sequences. PCR amplifications were carried out in a GeneAmp PCR System 2400 (Perkin-Elmer Cetus, Emeryville, CA). All experiments were done in parallel with positive and negative controls [Kwok and Higuchi, 1989].

PCR for Regulatory Region (RR)

A nested PCR was performed using two pairs of primers that anneal to the regions flanking the regulatory regions of BKV and JCV. Primers BKTT1(+) and BKTT2(–) were used to generate a 748-bp fragment for BKV and a 724-bp fragment for JCV after 40 amplification cycles in the first-round PCR [Flaegstad et al., 1991]. The second pair of primers [BK1(+) and BK2(–) for BKV; JC1(+) and JC2(–) for JCV] was designed to amplify a portion of the first-round PCR product [Markowitz et al., 1993]. The samples were subjected to 29 amplification cycles and the expected BKV and JCV products were 354 bp and 308 bp in length, respectively. The sequences and the nucleotide positions of the primers are reported in Table I. PCR products were

TABLE II. PCR Results for BKV in 15 Cases of Abortion

Case	Week of pregnancy	Reason for termination	PCR results (sample/region tested)					
			Placenta		Fetal brain		Fetal kidney	
			RR	VP1	RR	VP1	RR	VP1
1	23	Trisomy 18	–	–	–	–	–	–
2	20	Hydrocephalus	+	–	+	–	+	+
3	14	Trisomy 18	–	–	–	–	–	–
4	16	Polycistic kidney	+	–	+	–	+	–
5	24	Hydrocephalus	+	+	+	–	+	+
6	20	Hydrocephalus	+	–	+	–	+	–
7	22	Arnold-Chiari	–	–	–	–	–	–
8	20	Arnold-Chiari	+	–	+	–	–	–
9	24	Trisomy 18	+	–	+	–	–	–
10	23	Hydrocephalus	+	–	+	–	+	–
11	20	Hydrocephalus	+	+	+	+	+	–
12	22	Hydrocephalus	+	–	+	–	+	–
13	24	Trisomy 21	+	–	+	–	–	–
14	23	Arnold-Chiari	+	+	+	–	+	–
15	23	Polycistic kidney	+	+	+	–	+	+
Total			12/15	4/15	12/15	1/15	9/15	3/15

detected by ethidium bromide staining after electrophoresis on 3% agarose gel.

PCR for VP1 Region

PCR primers were chosen to anneal to the region flanking the VP1 subtype-specific region of BK virus [Jin et al., 1995]. Primers VP1-7(+) and VP1-2R(–) (see Table I) were expected to generate a 579-bp fragment for BKV after 35 amplification cycles in the first-round PCR. Samples were subjected to 2 min of denaturation at 94°C, followed by 35 rounds of an amplification cycle consisting of 1 min at 91°C, 1 min at 55°C, and 1 min at 72°C, followed by one extension cycle consisting of 4 min at 74°C [Degener et al., personal communication].

The second pair of primers, 327-1(+) and 327-2(–), was designed to amplify a portion of the first-round PCR product. The samples were submitted to 35 amplification cycles and the expected BKV product was 327 bp in length [Jin et al., 1995]. The sequences of primers and the nucleotide positions are reported in Table I. The PCR products were detected by ethidium bromide staining after electrophoresis on 3% agarose gel.

Sensitivities of PCR for RR and VP1

The sensitivities of the two nested PCR assays for the RR and for the VP1 of BKV was estimated by amplification of serial dilutions of infected tissue culture fluids containing BK virus. Both PCR assays reached the same dilution points.

Sequencing of RR of BKV

PCR products were purified using QIAquick PCR purification kit according to QIAGEN protocol. DNA sequencing was performed by automatic DNA sequencer (370 A) according to the instruction of manufacturer (Amplicycle kit, Perkin-Elmer). In this procedure, 300–500 ng of BKV DNA and 0.8 pmol of primer BK2 or BK1 were used in each sequencing reaction.

Probe Preparation and Molecular Hybridization

PCR products (positive or negative for BKV) were determined by molecular hybridization test. To each sample a threefold volume of SSC (10×) and formaldehyde (6.15 M) was added and kept in ice for 5 min after denaturation at 65°C for 15 min. A plasmid containing the entire genome of BKV (PT strain) was used as a template in the nested PCR amplification for the RR in the presence of α^{32}P-dATP and α^{32}P-dCTP. About 4.5×10^6 cpm/ml was used in each hybridization mixture. The radiolabeled PCR product was purified following the instruction of the QIAquick PCR purification kit. The PCR products obtained from the different tissues were applied onto nitrocellulose filters by dot-blotting. Hybridization was performed under high stringency conditions as described by Sambrook et al. [1989].

Sequence Data Analysis

Sequence data were organized and analyzed using the Genetics Computer group sequence analysis software package [Devereux et al., 1984] on a VAX computer.

RESULTS

In this study, autopsy specimens collected from 15 aborted fetuses were investigated by PCR for the presence of polyomavirus DNA in maternal and fetal tissue. The RR DNA of BKV was detected in 12 (80%) samples of placenta, 12 (80%) samples of brain, and 9 (60%) samples of kidney (Table II). Nine cases showed the presence of BKV in all organs tested and three were positive in only placenta and brain. Only three cases (two embedded and one non-embedded in paraffin) were negative in all samples investigated. Out of the 12 cases positive for BKV RR, four placentas, one brain, and three kidney samples also showed the presence of BKV VP1 DNA. Six of 12 placentas in the control group were found to be positive for BKV RR DNA and four of

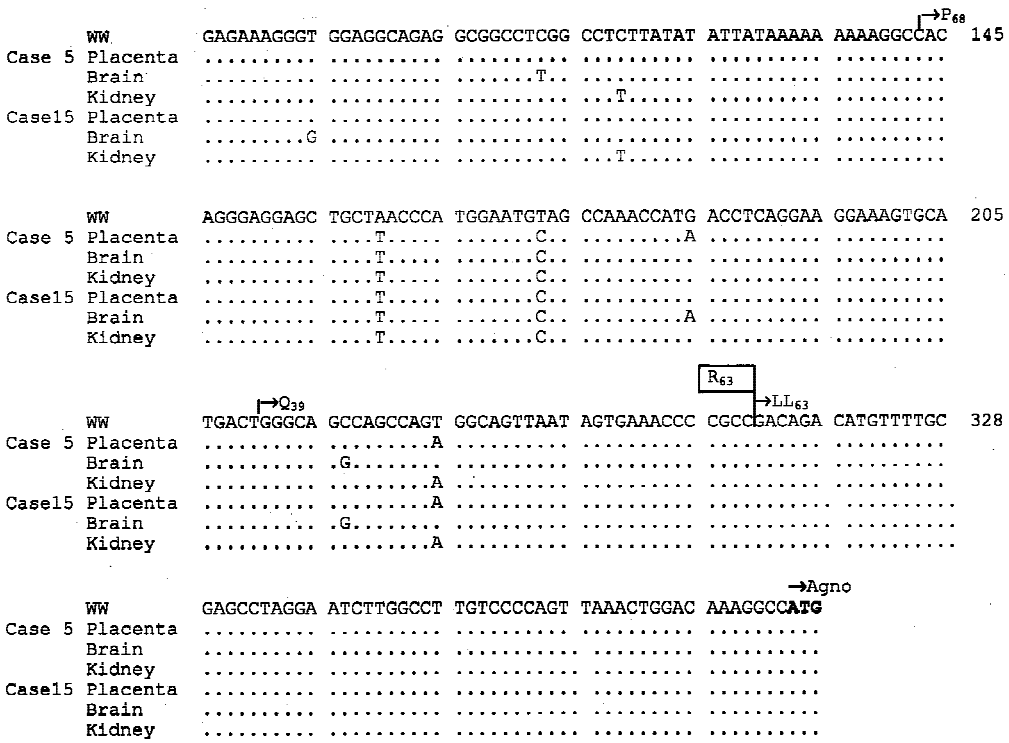

Fig. 1. Nucleotide sequences of the BKV regulatory region determined directly from the specimens of two cases. The sequence of archetype strain (WW) was shown for comparison and only differences were indicated. The regulatory blocks (P, Q, R, and LL) and the ATG initial codon for the agnoprotein are shown. The number system is that used by Seif et al. [1979].

these were also positive for the VP1 region. The sensitivity of the PCR for the RR and for the VP1 region was identical as shown by testing serial dilutions of the positive control. The molecular hybridization of the PCR products (positive or negative for BKV) with a radiolabeled probe confirmed only the positivity of those obtained by PCR. Each sample was also tested by PCR for the RR of JCV, but in none of the cases was JCV DNA found.

As it is known that a high degree of heterogeneity exists in the RR of BKV, the RR amplified from two cases, in which maternal placenta, fetal brain, and kidney were available, was sequenced to ascertain whether the same viral strain was present in both the maternal and fetal material. Compared to the nucleotide sequence of the archetype strain of BKV [Rubinstein et al., 1987], a characteristic deletion of the R_{63} box was found in all analyzed samples of both cases. Three to five nucleotide differences were also found between the tested samples and the archetype strain (Fig. 1).

DISCUSSION

Prenatal infection with BKV was suggested on the basis of reports of BK virus-specific IgM antibodies in children younger than two weeks of age and in umbilical cord blood [Taguchi et al., 1975; Rziha et al. 1978a]. However, a direct demonstration of maternal-fetal transmission has not yet been reported.

In the present study, the presence of viral DNA in both maternal and fetal specimens was demonstrated by means of PCR, with both the BKV regulatory and VP1 regions detected. The simultaneous presence of both regions of the BKV genome in maternal and fetal tissues provided strong evidence for transplacental BKV infection.

In order to ascertain whether the same virus strain was present in both maternal and fetal specimens, the RR of two cases were analyzed. The VP1 region was not sequenced since BKV strains with identical genotypes could show differences in the length of the RR [Jin and Gibson, 1996]. The sequences of the RR from the two cases showed only a limited number of point mutations (3–5) among the samples, without any difference in the length of the amplified RR fragments. The RR from all the samples shows a characteristic deletion of the R63 box comparing with the archetype strain WW [Rubinstein et al., 1987]. This is similar to the results found in the PBMC of HIV-positive and -negative subjects [Degener et al., personal communication]. However, size differences due to duplication or deletion of the blocks in the RR were seen in different BKV strains [Jin and Gibson, 1996].

Since high variability in the RR of BKV is known [Flaegstad et al., 1991; Jin and Gibson, 1996], the finding of similar sequences of the RR in different samples of the same case could be considered as evidence for maternal-fetal transmission.

The presence of BKV DNA in both maternal and fetal specimens in 12 out of 15 cases of abortion shows that there was no relationship between BKV infection and

the reasons for termination of pregnancy. Three (cases 1, 3, and 7) of the 15 cases were negative in all specimens for both the RR and the VP1 region by PCR (Table II). Specimens of cases 1 and 3 were embedded prior to PCR but those of case 7 were not. Therefore, there was no significant difference in PCR detection between the fixed and unfixed materials. The placentas from 6 of 12 women with a normal pregnancy outcome were also positive for BKV, suggesting that vertical transmission may be the predominant mode of transmission of BKV. It is not clear why there were significant differences between the detection of RR (60–80%) and VP1 (6.7–26.7%) sequences since the sensitivities of the RR-PCR and the VP1-PCR were identical using cell-cultured materials.

It is interesting to underline the different results obtained for JCV, since neither maternal nor fetal specimens were found positive for the DNA of this virus. Similar to BKV, in fact, JCV can also establish persistent infection and JCV DNA was detected in urine of pregnant women [Arthur and Shah, 1989; Flaegstad et al., 1991; Jin et al., 1995]. However, transplacental transmission of JCV was not found in our study.

Since the presence of BKV DNA was observed in a high percentage of maternal and fetal materials, the findings suggest that vertical transmission may be the predominant mode of transmission of BKV.

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